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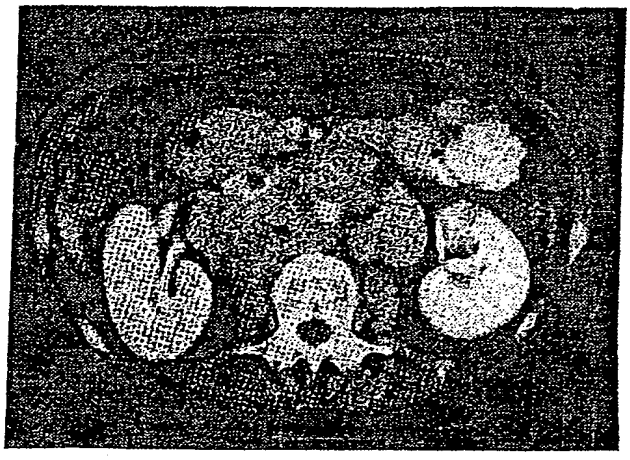

(57) Abstract

The invention relates to pharmaceutical compositions for therapeutic and/or diagnostic use comprising an antibody conjugate consisting of a monoclonal antibody with binding affinity to the CD30 cell surface antigen and a radioactive isotope linked to the antibody either directly or through a linker molecule or by means of a chelating agent. According to a preferred embodiment of the invention said antibodies are the murine antibody Ber-H2 or another antibody that recognizes the Ber-H2 epitope. Appropriate isotopes for the present invention are for example ^{99m}Tc, ^{111}In, ^{123}I, ^{131}I, ^{186}Re, ^{188}Re and ^{90}Y.

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ANTIBODY/RADIOISOTOPE CONJUGATE FOR TUMOR DIAGNOSIS AND/OR THERAPY

The invention relates to pharmaceutical compositions for therapeutic and/or diagnostic use comprising an antibody conjugate consisting of a monoclonal antibody with binding affinity to the CD30 cell surface antigen and a radioactive isotope linked to the antibody either directly or through a linker molecule or by means of a chelating agent.

The hybridoma technology for the production of murine monoclonal antibodies (muMAb) was introduced by Köhler and Milstein in 1975 (44). Starting with monoclonal anti-CEA antibodies (Mach et al., 1981), these radioimmunopharmaceuticals were applied in the clinic and more than a decade later, hundreds of different muMAb have been used in patients. This nuclear immunological approach has become an essential diagnostic tool, especially in the follow-up of colorectal and ovarian cancer, where the main indication of immunoscintigraphy is the possibility for an earlier detection of tumor recurrences in the follow-up.

Nowadays, immunoscintigraphy is regarded as an important adjunct

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to conventional imaging procedures such as CAT scan (CT), NMR or ultrasound (US). Immunoscintigraphy can also be applied as "immunoscintimetry", i.e. for the quantification of tumor vitality expressed as tumor uptake or in evaluating therapy response. The diagnosis of primary tumors is not yet a main indication for immunoscintigraphy, and immunoscintigraphy is also not suitable for tumor screening.

The greatest practical progress during the last years was the efficient direct labelling of MAb with ^{99m}Tc, which does not only provide better images, but also drastically reduces the radiation dose to the patient. Thus, the routine clinical application of muMAb was made possible: ^{99m}Tc is always available, it has a short have life and ideal imaging properties and it is cheap.

Nevertheless, immunoscintigraphy had already been successful when using ^{131}I, ^{123}I or ^{111}In labeled antibodies. Despite the disadvantages as compared to ^{99m}Tc, ^{131}I and ^{111}In labelled antibodies were proven to be effective diagnostic agents in multicenter studies.

Aside from the advantages, the application of radiolabeled murine Mab also poses difficulties. Human anti-mouse antibodies (HAMA) are induced following application with effects on scintigraphic images and in vitro assays. Among more than 3000 patients injected with muMAb for the first time for diagnostic purposes, allergic reactions were only seen once (data from IRIST group) in Europe. From over 2,500 applications, only 2 patients showed a mild local allergic reaction after 3rd application of muMAb. A more frequent problem, however, are elevated HAMA titers (20% after first application, 50-100% after repeated application).

Existing cancer therapy is at present characterized by both relatively low efficacy and considerable toxicity. One promising approach to overcome these shortcomings has been to target the-

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rapy directly to the tumor cell with specific molecules such as monoclonal antibodies.

Great expectations were based on the use of radiolabeled Mab in cancer therapy. So far, these expectations could not be completely fulfilled. One of the limiting factors of MAb therapy is the insufficient target-epitope saturation after single applications. Repeated injections, however, do not necessarily solve this problem because of HAMA development.

A further complication is the difficult access of the radioimmunoconjugate to reach its target, which is especially true for solid tumors. On the other hand, radioimmunotherapy has shown significant therapeutic effects in non-solid cancers, especially in the treatment of hematological neoplasias, e.g. lymphomas and leukemias (35, 36).

Conventional imaging modalities comprise X-ray CT scan, sonography, magnetic resonance imaging (all morphologically orientated) and nuclear medicine methods like gallium-67 scintigraphy and skeletal and bone marrow scintigraphy as well as positron-emission tomography (PET using F-18-FDG).

All these methods are useful in many situations for imaging for example Hodgkin's disease, but they each have certain limitations with regard to sensitivity and specificity. CT and sonography are very sensitive but show a low specificity especially after treatment. Gallium scintigraphy has a low sensitivity in some areas and cannot differentiate between inflammatory changes and tumor involvement.

The problem which is underlying the present invention is to provide a pharmaceutical composition, which is used for detecting tumor cells and/or for tumor therapy in cases that are resistant to conventional therapy. The pharmaceutical compositions should have a high binding activity as well as a high

tumor specificity without showing cross-reactivity or toxicity to other tissues, respectively.

According to the present invention these problems are solved by a pharmaceutical composition comprising an antibody conjugate consisting of a monoclonal antibody with binding affinity to the CD30 cell surface antigen and a radioactive isotope linked to the antibody either directly or through a linker molecule or by means of a chelating agent.

The pharmaceutical compositions of the present invention are prepared for use as a diagnostic agent for the detection of tumor cells by radioimaging and/or for use as a therapeutic agent for the treatment of human malignances characterized by the expression of CD30 surface antigen.

It has been shown by the present invention that the disadvantages known from the prior art could be overcome by the use of highly specific anti-CD30 antibodies linked to a radioactive isotope. The pharmaceutical compositions of the present invention are characterized by the high binding affinity and the high specifity of the antibodies contained therein. Since significantly less or no cross-reactivity with other tissues is observed, no side-reactions occur with the compositions of the present invention.

The radioisotopes are linked to the antibodies by way of direct or indirect methods.

For example, chelating agents which are bound to the antibody are used to form strong complexes with the respective radioisotopes (40). The well known N_2S_2 technique (41) is another example for an indirect method which can be applied according to the present invention.

In order to directly link radioisotopes to antibodies thiol (SH)

groups of the antibodies are photoactivated (42) or chemically activated (43).

According to a preferred embodiment of the invention the antibodies of the pharmaceutical compositions are the murine antibody Ber-H2 or any other antibody that recognizes the Ber-H2 epitope.

These antibodies, especially Ber-H2, have been shown to combine all prerequisites, such as high binding activity, high specifity, lack of cross-reactivity with other tissues, low dosages etc., which are essential for an efficient tumor diagnosis and/or therapy without toxic side effects known from prior art methods.

The monoclonal antibody (mAb) Ber-H2 (CD30) antibody was briefly described in 1987 and in more detail in 1989 by R. Schwarting et al. The antibody is produced by the equally named Hybridoma, which has been deposited at the European Collection of Animal Cell Culture (ECACC), Public Health Laboratory Service, Porton Down, Salisbury, Wiltshire, U.K., under the conditions of the Budapest Treaty under the preliminary number Ber-H2 92012823 on January 28, 1992.

The Ber-H2 murine monoclonol antibody (mAb) (1) recognizes a fixative-resistant epitope of the activation antigen CD30 (Ki-1) (2), a glycoprotein of 120 kD which is strongly expressed on the surface of Hodgkin's (H) and Reed-Sternberg (R-S) cells of Hodgkin's disease (HD) (3,5), and all cells of the newly recognized category of high-grade non-Hodgkin's lymphoma, the anaplastic large cell (ALC) lymphoma (6,8). In normal human tissues, the Ber-H2 mAb only reacts with a small population of large cells preferentially localized around B cell follicles.

The restricted reactivity in vitro of Ber-H2 with normal and pathological tissues makes it an exceptionally useful tool for radioimmunotherapy of CD30-expressing neoplasms (e.g. HD and ALC

lymphomas) especially in conjunction with other therapeutic strategies and for those cases that are resistant to conventional therapy and for diagnosis.

For use of Ber-H2 as a diagnostic agent, it is crucial that the in vivo distribution of the monoclonal antibody after intravenous application is the same as the CD30 antigen, i.e. that the Ber-H2 will bind in vivo similarly specific to the CD30 antigen as in vitro. It is in fact well known that a number of important factors, including the stability, isotype and molecular size of the mAb, the presence of the target antigen in the serum, the size, sclerosis and vascularization of the tumors, the permeability of the vessels to the antibody, and the ability of the antibody to reach its target without being cleared by the liver or spleen may affect the in vivo distribution of a given mAb (9,10).

According to the present invention, it was surprisingly found that the Ber-H2 mAb fulfilled all essential prerequisites for its use as a diagnostic agent and consequently for its therapeutic application as a radiopharmaceutical. It is able to reach the tumor cells (Hodgkin and - Reed-Sternberg cells) in a sufficiently high concentration when given intravenously and it is unreactive with normal human tissues in vivo.

In particular, the present invention is based on clear evidence that: a) immunoscintigraphy with radiolabeled Ber-H2 mAb (CD30) allow the sites involved by Hodgkin's disease to be detected; b) when injected in vivo, the Ber-H2 mAb binds exclusively to H and RS, which is entirely consistent with the Ber-H2 staining pattern previously described in vitro using frozen sections; c) complete saturation of CD30 binding sites on H and RS cells in all sites involved by HD can be achieved; d) there are no side effects associated with the administration of unmodified Ber-H2 in the dosage used in this study; e) strong in vivo binding of the unmodified Ber-H2 to H and RS cells does not produce any

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anti-tumor effect.

According to the present invention, it was found that the majority of sites previously documented to be involved by HD by clinical examination, conventional techniques and/or histology, were visualized by ^{131}I-Ber-H2 scintigraphy. Immunohistological studies provided conclusive evidence that the radioactivity content and positive imaging was the result of the specific in vivo labeling of H and RS cells by the ^{131}I-Ber-H2 mAb. Despite the high specificity of in vivo targeting seen in our cases, however, the interpretation of positive images was sometimes inconclusive at planar scintigraphy and required confirmation by single photon emission computerized tomography (SPECT) and/or kinetic studies (T1/2 tumor higher than T1/2 heart). A possible explanation for the weak transmitted signal at scintigraphy is probably due to the fact that radiolabeled H and RS cells account for only a small percentage (about 10% in our cases) of the whole cell population (lymphocytes, eosinophils, macrophages, etc) constituting the tissues involved by HD. This would also explain the lack of imaging of lymph nodes showing, at immunohistological level, strong in vivo targeting by Ber-H2 in patients 2 and 4. In the latter cases, the small size (1.5 cm) of the involved lymph nodes may have also been responsible for the lack of visualization, as previously reported by others (28). The quality of Ber-H2 imaging of HD can be significantly improved by using other radioactive isotopes, in particular Technetium or Indium (29) and Rhenium instead of ^{131}I.

The disadvantage of ^{131}I is its high amount of β-emission. For imaging γ emission is required. The relatively long half-life of ^{131}I leads to a lower imaging quality.

Compared with the cheap ^{131}I, ^{111}In, which is produced in cyclotrons, is very expensive. The radioisotope can be linked to the antibody by chelating agents or other spacer molecules. It is the advantage of ^{111}In that the half-life of the radioisotope

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corresponds to the biological half-life of antibodies, which leads to an efficient use of the respective antibody conjugates.

^{99m}Tc is another radionuclide which can be used for radioimmunoimaging. It has a very short half-life and its costs are low, because it is a generator product. As the radiation dose is low, this radioisotope allows a relatively harmless handling or application in radioimmunoimaging, for the physician as well as for the patient. The difficult linker chemistry, i.e. the strong binding of the radioisotope to the antibody, is the only disadvantage when using ^{99m}Tc.

According to the invention it was immunohistologically demonstrated that, when injected in vivo, Ber-H2 is able to reach neoplastic cells in all body sites involved by HD and binds only to H and RS cells. In particular, there is no non-specific in vivo binding of Ber-H2 by normal cells bearing Fc receptors (e.g. monocytes, macrophages, granulocytes, follicular dendritic reticulum cells, etc.) in any of the human lympho-hemopoietic tissues studied (lymph nodes, liver, spleen and bone marrow). This finding is in line with the immunostaining pattern already described for the Ber-H2 mAb in vitro (1) and may be related to the low affinity of the Ber-H2 mAb (IgG1 subclass) for human IgE Fc receptors. In this regard, Ber-H2 differs from the prototype CD30 mAb Ki-1 (IgG3 subclass) that has been reported to react with activated macrophages (30). There was immunohistological evidence of in vivo binding of Ber-H2 to H and RS cells from the 24th hour (case 4) but it could have occurred even earlier. The antibody was still detectable on tumor cells 72 hours post-infusionem (case 2). Such an extended persistence of the antibody on the surface of the tumor cell is particularly useful from the therapeutic point of view.

The invention provides immunohistological evidence for the surprisingly high avidity in vivo binding of Ber-H2 mAb to H and RS cells, as demonstrated by the fact that a complete saturation

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could be reached at all in vivo CD30 binding sites on H and RS cells in all body sites involved in the disease (lymph nodes, spleen and bone marrow). The dose necessary was much lower than that reported to be necessary for good in vivo targeting of solid tumors (31) and other human lymphomas (32). In one immunohistological study (31), only incomplete saturation of antigenic sites was achieved on the surface of malignant cells in cutaneous metastases of melanoma with doses of up to 200 mg of murine anti-melanoma 9.2.27 mAb. Even larger doses (range 500 mg to 1.5 g) of anti-idiotype (32, 33), IF5 (anti-CD20) (34) or MB-I (anti-CD37) (35) mAbs have been required for good in vivo labeling of malignant cells in non-Hodgkin lymphomas of B cell type, and, despite the high dose, antigenic sites were only partially saturated. Surprisingly, according to the invention, the in vivo targeting of H and RS cells in the patients occurred under conditions considered to have a negative influence on the bio-distribution of mAbs within neoplastic tissues, e.g. lymph node or splenic or extranodal and extrasplenic "bulky disease" (cases 1 and 2) areas of necrosis in the spleen (case 2) and nodular sclerosing histology (case 3).

In contrast, the in vivo labeling of neoplastic cells in non-Hodgkin lymphomas of the B and T cell type by mAbs such as anti-idiotype (32, 33), T101 (CD5) (16), 1F5 (CD20) (34), MB-I (anti-CD37) (35) has been usually hampered by the high serum level of idiotypes or the high serum level and/or the wide expression of the CD5, CD37 and CD20 molecules on normal T or B cells. In consequence, efficient in vivo targeting of neoplastic cells in non-Hodgkin lymphomas can only be obtained by injecting large doses of mAbs as mentioned above.

As the results presented here were obtained under most disadvantageous conditions, e.g. presence of "bulky disease" (cases 1 and 2), HD cases rich in H and RS cells, and at stages III or IV, similar and even lower amounts of Ber-H2 mAb are sufficient to efficiently label H and RS cells in prognostically more fa-

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vourable categories of HD.

The immunohistological results obtained according to the invention provide the rational basis for using the Ber-H2 mAb as a therapeutic agent for CD30-expressing neoplasms (e.g. HD and ALC lymphomas) in conjunction with other therapeutic regimens and especially for those cases that are resistant to conventional therapy. Hodgkin's disease is an ideal model for this purpose since: a) the percentage of neoplastic cells is relatively low; b) the Ber-H2 mAb does not significantly cross-react with normal human tissues and c) most cases of HD are highly radiosensitive. According to preferred embodiments of the invention conjugates of the Ber-H2 mAb with either ^{131}I, ^{90}Y (36), ^{212}Bi (37) or ^{186}Re, ^{188}Re are particularly suited for radioimmunotherapy. The findings according to the present invention demonstrate that such a therapeutic effect can be obtained by administering low doses of Ber-H2 immunoconjugates.

As γ radiation is required for radioimaging, ^{90}Y, which is a pure β emitter can only be applied for therapeutic uses. Because of its high energy, lower doses of antibody conjugates are necessary and the depth of penetration in tissue (6 mm) can be of use in some applications. Since yttrium is osteophilic, the problem associated with the use of ^{90}Y is its toxicity for bone marrow. It is therefore very important to assure that ^{90}Y is strongly linked to the antibody in order to prevent any liberation of the radionuclide.

In preferred embodiments of the invention rhenium isotopes are utilized. Wheras ^{188}Re as a generator product is very cheap, ^{186}Re is much more expensive. Because of their relatively low specific activity comparatively higher doses of rhenium isotopes are required for therapy as compared to yttrium.

However, rhenium isotopes can advantageously be utilized in the form of an antibody conjugate both for therapeutic and imaging

(i.e. diagnosis or quantification) applications without the need of subjecting the patient to separate injections of antibody conjugates for diagnosis and therapy.

Although the pharmaceutical compositions according to the present invention may be applied as pure antibody preparations, the conjugates can be formulated together with suitable pharmaceutically acceptable carriers and aiding substances, respectively. The pharmaceutical compositions are in a suitable form for injection.

Preferably 1 to 2 mg of antibody cojugate are utilized for imaging and 10 to 50 mg per single injection are used for therapy depending on the specific activity.

The invention is further described below by way of examples, wherein the in vivo targeting of H and RS cells was assessed in four patients by two different methods: a) radioimaging of the tumor following injection of a diagnostic dose of ^{131}I-labeled Ber-H2 mAb; and b) immunohistological analysis of tissue sections, for direct evidence of antibody localization on the tumor cell membrane.

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The following Figures are referred to in the Examples:

Figure 1 is an abdominal CT scan of Patient 1 which shows large lymph node masses between the two kidneys.

Figure 2 is a SPECT (single photon emission computerized tomography) taken at the same abdominal level as Fig. 1. Lymph node masses (T) are imaged by ^{131}I Ber-H2 (72 hours after the infusion).

Figure 3 is an anterior view of the head 48 hours following Ber-H2 infusion (Patient 3). A left neck lymphoma mass (T) is clearly imaged (* indicates thyroid uptake).

Figure 4 is a left neck lymph node biopsy from the same patient as Fig. 5, taken 72 hours following Ber-H2 infusion. a) Frozen section incubated only with rabbit anti-mouse antiserum and developed by the APAAP technique. Clusters of H and RS cells encased by fibrous tissue are strongly labeled in red. Notice the thickened node capsule (C) (hematoxylin counterstain; x 125); b and c) Higher power views of the same field as Fig. 6a showing strong surface in vivo targeting of tumor cells surrounded by fibrous tissue (6c) (hematoxylin counterstain; x 500).

Figure 5: The spleen removed at staging laparotomy shows massive involvement by HD (Patient 2).

Figure 6: Frozen section from the spleen nodules shown in Fig. 5 following incubation with rabbit anti-mouse antiserum and developed by the APAAP technique (*Indicates a central arteriole. Hematoxylin counterstain; x 400).

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Patients and Methods

Patients:

Four patients with histologically confirmed (11), advanced HD in relapse were investigated (see Table 1). In all cases, H and RS cells in lymph node biopsies taken at initial diagnosis displayed strong surface reactivity with the Ber-H2 (CD30) mAb. This was the sine qua non condition for inclusion in the study. All patients received a diagnostic dose of ^{131}I-labeled Ber-H2 mixed with scaled-up quantities of "cold" Ber-H2 (see below). New tumor biopsies were obtained from three patients for immunohistological evaluation of the in vivo binding of Ber-H2 to neoplastic cells 1 to 3 days following mAb injection. The study was approved by the local ethical committee. All patients gave informed consent for the above procedures.

Example 1

Purification of the Ber-H2 monoclonal antibody:

The Ber-H2 mAb (mouse IgG1) was purified from conventional hybridoma culture supernatant by affinity chromotography on protein A-sepharose CL-4B, as previously described (12). The antibody was eluted with 0.5 M citrate buffer pH 5.0, and dialysed against 0.01 M PBS, pH 7.2. The antibody preparation was more than 98% pure as determined by cross immunoelectrophoresis and SDS-PAGE analysis. After ultracentrifugation to remove microaggregates, the preparation was passed through a 0.2 µm filter (Millipore) to ensure sterility, and stored at -70 °C prior to use. The purified antibody passed the safety control tests as established by the document entitled "Empfehlungen für die Herstellung und Prüfung in vivo applizierbarer monoklonaler Antikörper" (13). In particular, the antibody preparation was shown to be free from bacteria, fungi and adventitious viruses. The absence of murine viral contaminants was demonstrated by the mouse antibody production (MAP) test, in which contamination with 16 murine viruses was evaluated by injection of the test article into mice and determination of antibody production to the viruses of interest. The Limulus Amebocyte Lysate assay for endotoxin was negative. The total content of DNA per dose was less than 10 picograms.

Example 2

Radiolabeling of Ber-H2:

All radiolabelling procedures were performed at the Radiopharmaceutical Division of Sorin Biomedica, Saluggia (VC), Italy. The Ber-H2 mAb (IgG1 subclass) was radioiodinated using the Iodogen method (Pierce Rockford IL). The antibody (2 mg in 0.33 ml PBS at pH 7.4) was added to a reaction vial previously coated with

100 µg Iodogen. $Na^{131}I$ (8 mCi:296 MBq) of $Na^{131}I$ was added to the vial and the reaction mixture incubated for 15 min. at room temperature under magnetic stirring. The radiolabeling efficiency was assayed by ascending chromatogrophy on silica gel plate (eluant methanol/water 85:15). The mean labeling yield was >95.0%. The unincorporated ^{131}I was separated by anion exchange chromatography through a syringe containing 1 - 2 ml Dowex AG 1X8 resin (Biorad) and eluted with 3 ml 0.3% HSA 0.04 M phosphate buffer at pH 7.4. After iodination, the Ber-H2 mAb retained more than 70% of its baseline immunoreactivity, as shown by cell binding (15) and frozen section assays. The final product was sterile and free of pyrogens.

Example 3

Antibody administration:

An intradermal hypersensitivity test was performed in each patient 1 hour before mAb administration with 20 µl of 1 µg/ml of the Ber-H2 antibody preparation in sterile buffered saline. The site of injection was observed for 15 min. No positive reaction was seen. To minimize thyroid uptake of radioiodine, patients were treated with a saturated solution of potassium iodide (10 drops, 3 times per day) from 2 days before injection until 10 days after (16).

Patient 1 received 0.5 mg ^{131}I-Ber-H2. Studies of scaled-up doses were performed by co-injection of the other patients with 14 mg, 29 mg, or 39 mg unlabeled Ber-H2 mAb and 1 mg ^{131}I-Ber-H2 for a total dose of approximately 15 mg, 30 mg and 40 mg respectively (Table 1).

Table 1: Patient data

Patient (Age/Sex)	Restage*	Histol. type	Expression of CD30	Dose+ mAb	Specific activity
1. 36/F	IVB* (CS)	HD-NS	Strong	0.5	7.8
2. 52/F	IIIB (PS)	HD-MC	Strong	15.0	0.2
3. 33/F	IIA* (CS)	HD-NS	Strong	30.0	0.3
4. 58/M	IVB (CS)	HD-MC	Strong	40.0	0.12

+ Total of labelled and cold antibody

* At the time of relapse.

Cs: Clinical staging; PS: Pathological stating; BMT: Autologous bone marrow transplantation; IL-2: high dose interleukin-2 therapy; HD: Hodgkin's desease; NS: Nodular sclerosing; LD: lymphocyte depletion; MC: Mixed cellularity.

The 0.5 mg dose of radiolabeled Ber-H2 was injected over 10 min. Higher doses of the antibody were given as a continuous 3-hour infusion in 500 ml of normal saline containing 5 % human serum albumin. Patients were routinely premedicated with 10 mg of clorfeniramine 30 min. before antibody administration. Vital signs were recorded frequently during antibody infusion and the following 12 hours. No hypersensitivity reaction occurred. Complete laborotory investigations were repeated on days 1 and 7 following infusion.

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Example 4

Radioimmunodiagnostic studies:

Whole body multiple anterior and posterior images (four minutes per image) were obtained within two hours and daily for up to six days after the infusion using a large-field-of-view gamma scintillation camera (GE maxicacmera/37) with a high energy collimator (400 keV maximum). Patient 1 was subjected to single photon emission computerized tomography (SPECT) 48 and 72 hours post-infusion. The data were stored and analyzed in an interfaced computer system (MEDUSA, Sepa) which generated digital images. Scans were read independently by two nuclear medicine physicians who had no prior knowledge of disease sites.

The in vivo distribution and uptake-disappearance curves of ^{131}I-Ber-H2, using regions of interest analysis (thyroid, lungs, heart, liver, spleen, kydney and lymph nodes) was determined by the geometrical mean method of count rates corrected for decay and background and expressed as a percentage of whole body reactivity (17-19).

Blood samples were obtained at 0.083, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 18, 24 hours, and then daily for up to six days. In addition, all urine and faeces were collected daily for up to six days after antibody administration. Plasmatic, urinary and faecal radioactivity were measured by counting an aliquot of each in a NaI(Tl) well detector interphased to a multichannel analyzer. Nonlinear regression calculations were then performed by Marquardt's method to determine standard pharmacokinetic parameters.

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Example 5

Tissue handling:

Tumor was not accessible for biopsy in patient 1. Patient 2 underwent a re-staging exploratory laparotomy 72 hours following infusion of the radiolabeled antibody. Intraoperatively, the spleen and several abdominal lymph nodes were removed; wedge liver biopsies and a Jamshidy bone marrow biopsy were also performed. Patients 3 and 4 had a lymph node biopsy performed 48 and 24 hours respectively following injection of mAb.

All samples were obtained fresh and placed in normal saline to remove any excess blood; thereafter, they were weighed and counted for radioactivity using gamma scintillation spectrometry. A radioactive standard was counted in order to correct relative tissue counts to absolute counts and the results expressed in uCi/g of tissue. Tissue samples were then split, one portion being B5-fixed and paraffin-embedded for routine diagnosis and the other snap-frozen in liquid nitrogen and cut at 5 µm in a cryostat.

Example 6

Immunohistological analysis:

Immunohistological studies were performed on frozen tissue sections that had been previously air-dried overnight at room temperature and fixed in acetone for 10 min (21, 22). In vivo targeting of the Ber-H2 mAb to H and RS cells was assessed by direct incubation with the secondary rabbit antimouse Ig followed by APAAP complexes (23). In order to avoid any cross-reactivity with human immunoglobulins, the secondary antibody was preincubated with 10% normal human serum 1 hour before the immunostaining procedure. Frozen sections from lymph node biopsies removed

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at the time of initial diagnosis (e.g. before mAb infusion) incubated with secondary rabbit antibody served as a negative control, and frozen sections from pre- and post-mAb infusion lymph node biopsies incubated with Ber-H2 as a positive control. Other controls included APAAP staining of frozen sections with the following mAbs (24): CD3 (UCHT1), CD20 (L26), Ber-Mac 3 (anti-macrophage), and Ki-67 (anti-proliferating cells) (25).

Example 7

Serum assays:

Pre-infusion serum levels of soluble CD30 molecule in the serum were determined in all patients by an ELISA technique, as previously described (26).

Example 8

Results of the Radioimmunodiagnostic studies:

In Table 2, the results of organ involvement as revealed in four patients by CT, radioimaging, histology and APAAP-immunohistology are compared. The neoplastic sites involved were usually visualized by immunoscintigraphy 24 hours following injection of the labeled mAb in all cases. However, optimal images were seen at 48-72 hours, when blood background activity had decreased.

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Table 2: Correlation between clinical staging, imaging, histology, immunohistology and radioactivity content in four HD patients injected with ^{131}I-BerH2 mAb

Patient	Clinical staging	Imaging (time)*	Studies post-BerH2 injection		
			Histology* (% H & RG)	APAAP**	Radioact.
Case 1					
LN abdomen	+ (CT)	+ (72h)	na	na	na
Lung	+ (CT)	- (72h)	na	na	na
Spleen	- (CT)	+ (72h)	na	na	na
Liver	- (CT)	+ (72h)	na	na	na
Case 2					
LN med./hil.	+ (CT)	+ (72h)	na	na	na
LN abdomen	- (CT)	- (72h)	HD-MC (15%) ^	+	$9x10^{-4}$
Spleen	- (CT)	+ (72h) +	HD-MC (15%) ^	+	$1x10^{-3}$
Liver	- (CT)	+ (72h) +	Normal ^	-	$9x10^{-4}$
BM	-	- (72h)-	Normal ^	-	$5x10^{-4}$
Case 3					
LN neck	+ (CE)	+ (48h)	HD-NS (10%)	+	na
LN mediast.	+ (CT)	E (48h)	na	na	na
Liver	- (CT)	+ (48h)	na	na	na
BM	-	- (48h)	na	na	na
Case 4					
LN neck	+ (CE)	+ (24h)	HD-MC (15%)	+	$1x10^{-3}$
LN med.	+ (CT)	E (72h)	na	na	na
LN paraortic	+ (CT)	+ (72h)	na	na	na
LN iliac	+ (CT)	+ (72h)	na	na	na
Spleen	- (CT)	+ (72h)	na	na	na
Liver	- (CT)	+ (72h)	na	na	na
Bm	-	- (72h)	HD-MC	+	$1x10^{-3}$
Skin		- (72h)	Normal	-	$6x10^{-4}$
Adipose Tissue		- (72h)	Normal	-	$1x10^{-4}$

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BM: Bone marrow; CE: clinical examination; CT: Computed tomograph; med./hil.: mediastinal and hiler; H & RS: Hodgkin and Reed-Sternberg cell; HD: Hodgkin disease; NS: Nodular sclerosing; MC: Mixed cellularity; n: not available; E: equivocal image due to the high blook background.

* Refers to the optimal imaging time post-BerH2 injection.

** Refers to endogeneously bound Ber-H2 to H & RG cells after vivo injection of the antibody.
Staining was performed on frozen sections as indicated in Materials and Methods.

^ All samples (including a third bone marrow biopsy) taken during exploratory laparotomy.

Percent of the injected dose per gram of tissue.

Pre-infusion bone marrow biopsies; post-infusion bone marrow biopsy.

Patient 1 had a large (10 cm) mass and multiple pulmonary nodules. The mass in the abdomen was visualized by ^{131}I-Ber-H2 and was clearer with SPECT than with planar scintigram (Figs. 1 and 2). However, lung lesions were not detected by immunoscintigraphy.

Patient 2 had pathologically changed abdominal lymph nodes that were regarded as normal at CT, suspicious at lymphangiography, and were not positively imaged by ^{131}I-Ber-H2. Enlarged mediastinal and hilar lymph nodes at CT scan were also documented by immunoscintigraphy.

Patient 3 had several enlarged lymph nodes (2.5 cm) in the left supraclavear region. Optimal imaging of nodes in the neck was obtained 48 hours following ^{131}I-Ber-H2 injection (Fig. 3). As a control, no targeting of the neck nodes was observed following

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injection of the patient with a ^{131}I-labeled unrelated monoclonal antibody (anti-CEA) of the same subclass as Ber-H2. Slightly enlarged mediastinal lymph nodes at CT scan were not clearly imaged by ^{131}I-Ber-H2.

Patient 4 had a small lymph node (1.5 cm) in the left laterocervical region and several enlarged lymph nodes (3 cm) in paracortic and iliac regions. At the time when the node in the neck was removed for immunohistological studies (24 hours following injection of ^{131}I-Ber-H2), there was no positive imaging. Abdominal lymph nodes were well visualized by ^{131}I-Ber-H2 at 72 hours.

Non-specific liver and spleen uptake was seen in all patients. Thyroid and urinary bladder uptake of ^{131}I-Ber-H2 in all injected patients indicates in vivo dehalogenation. Thyroid uptake occurred in spite of the administration of Lugol solution, but did not cause hypothyroidism in any of our patients. A similar degree of liver, spleen and thyroid uptake has been reported in other studies that used ^{131}I-labeled mAbs for the imaging of cutaneous T cell lymphomas (16) and non-Hodgkin lymphomas of B cell type (19).

The results of region interest analysis for the various organs demonstrated that the maximum uptake for all was reached during the first 2 hours following injection. Table 3 summarizes the intercept (Ao) and the half time (T1/2) obtained from both single organs and tumor by a monoexponential model non-linear regression analysis. Biexponentially calculated pharmacokinetic parameters are given in Table 4.

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Table 3: Patient organ and tumor uptake, and biological clearance T1/2

	Patients							
	1		2		3		4	
	Ao*	T1/2 (h)	Ao*	T1/2 (h)	Ao*	T1/2 (h)	Ao*	T1/2 (h)
Heart	5.5	34.8	4.1	19.4	5.6	28.6	4.4	24.3
Lung	6.1	36.4	nd	nd	10.4	21.2	5.1	27.0
Liver	11.0	26.9	9.1	16.4	7.0	28.6	8.4	20.0
Spleen	2.6	41.7	1.9	18.4	2.1	35.8	3.0	23.1
Kidneys	1.7	76.3	1.6	19.1	2.0	30.5	0.8	32.6
Tumor								
Abd. mass	5.7	52.5	-	-	-	-	-	-
LN neck	-	-	-	-	1.1	51.7	-	-
LN med.	-	-	-	-	-	-	0.6	28.0
LN hilum	-	-	4.7	27.0	-	-	-	-
LN paraort.	-	-	-	-	-	-	0.3	37.7
LN iliac	-	-	-	-	-	-	0.9	38.4

* % injected dose

Abd.: abdomen; med.: mediastinum

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Table 4: Plasma Kinetic parameters

Patient	T1/2 h	T1/2 h	AUC*	Plasma/Cl. ml/min/sqm	Vdes L/sqm	Vc L/sqm
1	3.9	47.2	522.9	0.644	2.5	1.5
2	3.4	36.9	15775	0.657	1.9	1.1
3	5.7	73.2	30576	0.608	3.6	2.1
4	3.0	32.5	18764	1.156	3.0	1.9

*= AUC O-inf (ug per min per ml)
T1/2= Half time; AUC O-inf= Integration of the area under the plasma concentration curve; VDss= Apparent distribution volume at the steady state; Vc= Apparent volume of the central compartment.

Example 9

Histological and immunohistological studies:

No tissue specimens were available from patient 1 for immunohistologic studies after injection of the antibody. In patient 2, H and RS cells in the spleen and abdominal lymph nodes removed at restaging laporotomy (performed 72 hours following the injection of mAb) were specifically labeled on the surface by the injected Ber-H2. All neoplastic cells present in HD involved tissues, including those located at the border of large necrotic areas in the spleen, were targeted in vivo by Ber-H2. CD30 binding sites on H and RS cells were not completely saturated by the 15 mg injected dose of mAb, since incubation of adjacent sections with the mAb Ber-H2 increased the immunostaining intensity. Cell populations from uninvolved portions of spleen and lymph nodes showed no antibody binding. In spite of positive

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imaging, the liver was not histologically involved by HD and no Ber-H2 binding was detected in the organ at immunohistological level. In consequence, the exact localization of ^{131}I-Ber-H2 in the liver could not be established.

Patient 3 had a clinically involved left latero-cervical lymph node, that was also positive on scanning, removed 48 hours following Ber-H2 infusion. The histological pattern was that of a nodular sclerosis HD rich in H and RS cells. Direct incubation of frozen lymph node sections with secondary rabbit anti-mouse immunoglobulin antibody followed by APAAP complexes gave strong surface staining of H and RS cells (Fig. 4 a,b). All neoplastic cells in the different areas of the frozen sections, including those encased by bundles of fibrous tissue (Fig. 4c), were labeled, indicating in vivo bound Ber-H2. Complete saturation of the CD30 binding sites on H and RS cells was achieved following injection of 30 mg mAb. A similar immunohistological staining pattern was observed in the lymph node and bone marrow biopsies taken from patient 4, 24 hours following Ber-H2 injection.

The following common findings were documented in all patients. The staining pattern was distinct from the distribution of blood vessels within the tumor. In vivo labeling was highly selective, since no other cell constituents but H and RS cells were stained by the secondary antibody alone in frozen sections. APAAP staining of serial frozen sections with the Ber-H2 mAb gave an identical reactivity pattern, indicating that all CD30 binding sites of all neoplastic cells were fully saturated in vivo, as desirable for therapeutical applications by Ber-H2 at a dosage of 30 - 40 mg. As expected, incubation of frozen section with a number of primary mAbs, other than Ber-H2, resulted in the APAAP labeling of both H and RS cells (due to in vivo targeting of Ber-H2) and the cell population (e.g. macrophages) identified by the corresponding mAb in vitro. Hodgkin and RS cells in lymph node specimens taken at the time of initial diagnosis of HD (e.g. before the infusion of the mAb) failed to

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stain with secondary antibody and served as negative control.

Previous reports suggesting that RS cells usually contain passively absorbed polyclonal plasma immunoglobulins (27) raised the question as to whether the labeling of our patient's H and RS cells was the result of non-specific in vivo uptake of the injected Ber-H2 mAb mediated by the Fc fragment rather than through CD30-specific antigen binding sites. However, the first possibility was excluded because: a) the in vivo labeling pattern was identical to that produced by the Ber-H2 antibody in vitro (e.g. surface staining of all H and RS cells), whereas other cells, including those carrying IgE Fc receptors like granulocytes, macrophages etc., were totally negative (27); b) involved lymph nodes were not imaged following injection of an unrelated ^{131}I-labeled antibody (directed at the CEA antigen) of the same subclass as Ber-H2.

Example 10

Clinical effect and toxicity of the unmodified and/or inconjugated Ber-H2 monoclonal antibody:

There was no measurable decrease in the size of involved sites during the interval (range 15 to 40 days) between Ber-H2 mAb infusion and beginning of salvage chemotherapy in any of our patients. No toxic effects or laboratory abnormalities were documented for any of the Ber-H2 doses given.

Example 11

Serum assay:

Pretreatment CD30 serum levels were as follows: patient 1 (400 U/l), patients 2 and 3 (not detectable), patient 4 (100 U/l).

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Claims

1. A pharmaceutical composition for therapeutic or diagnostic use comprising an antibody conjugate consisting of a monoclonal antibody with binding affinity to the CD30 cell surface antigen and a radioactive isotope linked to the antibody either directly or through a linker molecule or by means of a chelating agent.

2. A pharmaceutical preparation according to claim 1, wherein the said antibodies are the murine antibody Ber-H2 or an antibody that recognizes the Ber-H2 epitope.

3. A pharmaceutical preparation according to claims 1 or 2, wherein said radioactive isotope is either iodine (I), yttrium (Y), indium (In), technetium (Tc) or rhenium (Re).

4. A pharmaceutical preparation according to claims 1 to 3 formulated for use as a diagnostic agent for the detection of tumor cells by radioimaging.

5. A pharmaceutical preparation according to claims 1 to 3 formulated for use as a therapeutic agent for the treatment of human malignances characterized by the expression of CD30 surface antigen.

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Figure 1

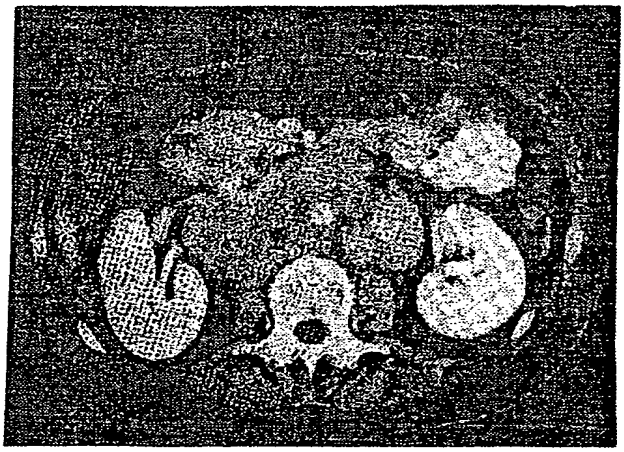

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Figure 2

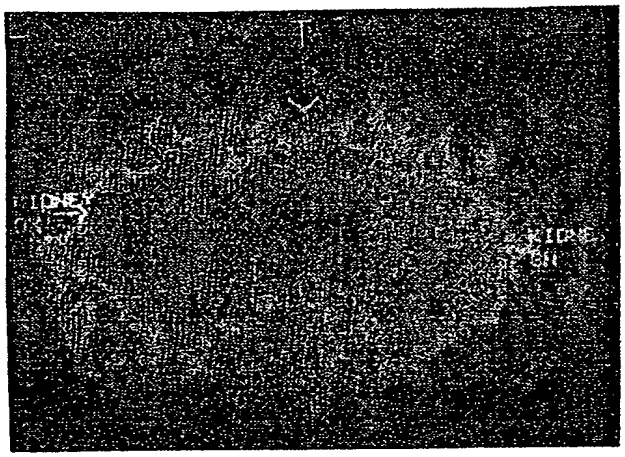

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Figure 3

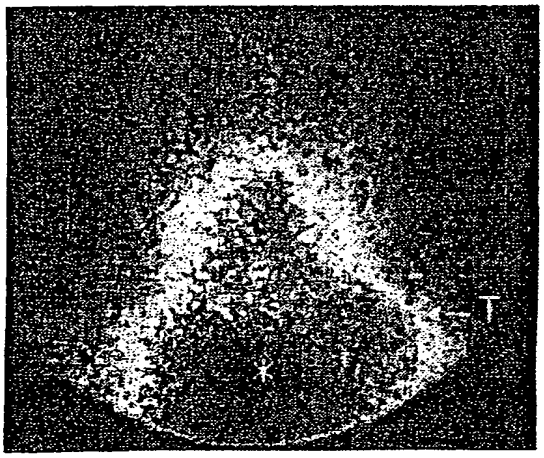

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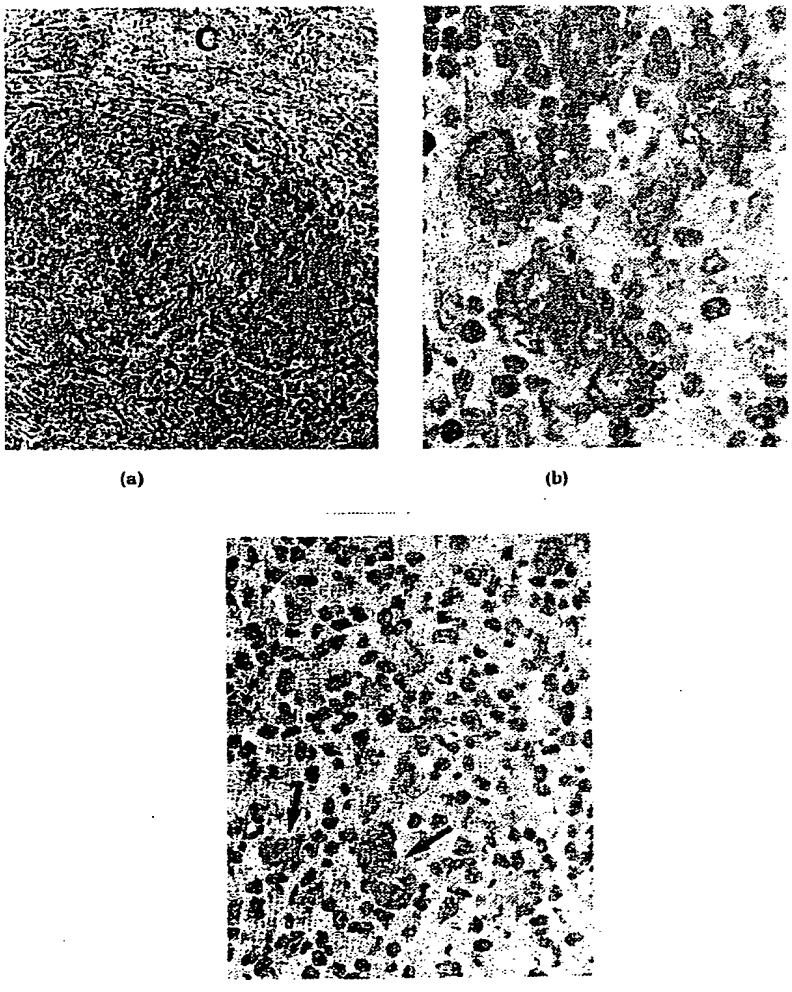
Figure 4



(a) (b)

(c)

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Figure 5

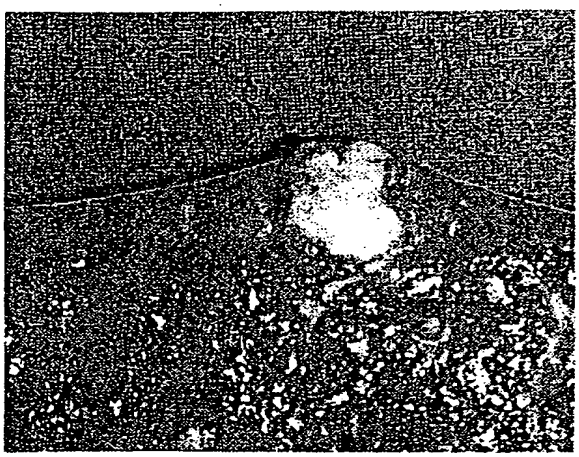

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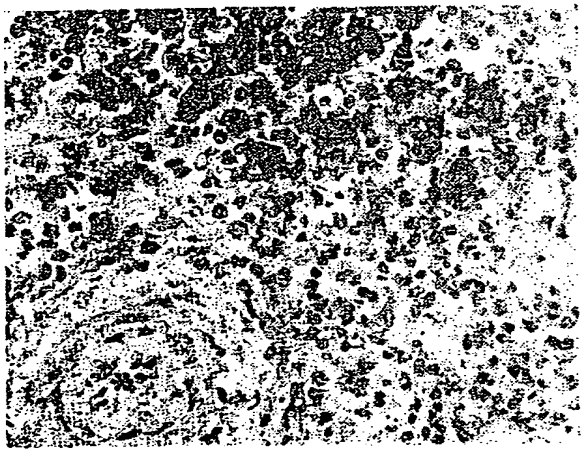
Figure 6



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INTERNATIONAL SEARCH REPORT

Internat. Application No
PCT/EP 93/02293

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K43/00 A61K49/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	WO,A,91 07437 (D. PARKER) 30 May 1991 see page 22, line 6 - line 26; figures 1,3 see page 27, line 15 - line 29 see page 39, line 19 - line 29; table I see page 43, line 16 - line 27 ---	1-5
X	DATABASE MEDLINE US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US AN=90067476 see abstract & ONKOLOGIE vol. 12, no. SUP1 , August 1989 pages 30 - 33 M.PREUNDSCHUH ET AL. 'IN VIVO IMAGING OF HODGKIN'S LYMPHOMEN MIT MONOKLONALEN ANTIKORPERN.' --- -/--	1-5

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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Date of the actual completion of the international search	Date of mailing of the international search report
27 December 1993	

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Berte, M

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INTERNATIONAL SEARCH REPORT

Internat Application No
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(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE BIOSIS BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US AN=92:525938 see abstract & BR. J. HAEMATOL. vol. 82, no. 1 , 1992 pages 38 - 45 B. FALINI ET AL. 'IN-VIVO TARGETING OF HODGKIN AND REED-STERNBERG CELLS OF HODGKIN'S DISEASE WITH MOAB BER-H2 CD30 IMMUNOHISTOLOGICAL EVIDENCE.'	1-5
X,Y	DATABASE MEDLINE US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US AN=93081379 & ANN. ONCOL. vol. 3, no. SUP4 , September 1992 pages 53 - 57 DA COSTA ET AL. 'IMMUNOSCINTIGRAPHY IN HODGKIN'S DISEASE AND ANAPLASTIC LARGE CELL LYMPHOMAS: RESULTS IN 18 PATIENTS USING THE IODINE RADIOLABELED MOAB HRS-3.'	1-5
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INTERNATIONAL SEARCH REPORT

Information on patent family members

Internation application No
PCT/EP 93/02293

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9107437	30-05-91	AU-A- 6909191	13-06-91
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